IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Wolfgang M. Franz et al.
Serial No. 09/068,751
filed: November 14, 1996
for: Gene Therapeutic Nucleic Acid Working Model and Its Production and Use for Treating Heart Disease

DECLARATION UNDER RULE 132

I, the undersigned, Wolfgang M. Franz, a citizen of Germany, do hereby depose and declare:

I have graduated in medicine and I was awarded the degree of PhD from the Technical University of Munich, Germany, in 1986.

My major research interest is directed to gene therapy of cardiovascular diseases.

I am co-inventor of the subject matter of U.S. Patent Application No. 09/068,751.

I have carefully read the Office Action dated December 20, 2001 and the specification of the present patent application.

With regard to the Examiner's contention that the specification does not enable any person skilled in the art to make and / or use the invention commensurate in scope with the present claims I would like to provide the following response:

The present application is, to my opinion, the first document disclosing the successful cardiac specific, in particular ventricle specific, gene transfer in neonatal or adult animals by making use of viral vector systems.

In my opinion, a skilled person would have been enabled by the detailed disclosure of the specification to perform the invention, in particular the tissue specific gene transfer in adult or neonatal animals.

The experiments disclosed in the specification illustrate operability of a **functional** regulatory nucleic acid sequence as obtained from the **rat** mlc-2 gene under conditions of somatic gene transfer (see in particular Examples 8 to 11 of the specification).

Meanwhile we were able to locate the corresponding regulatory sequence of the **human** mlc-2 gene. The obtained nucleotide sequence is attached as

ANNEX I

We have analysed the sequence for potential regulatory elements and could locate sequence motifs **almost identical** to those disclosed in the present specification and considered as vital for the intended purpose. These elements are, in 5'-3' direction, the following: CSS-like sequence, MLE1-box, HF-1a-, HF1b-, HF-2-, HF-3- and E-box. The same sequence motifs have been identified in the specification to play a critical or at least favourable role in the regulation of cardiac specific gene expression.

Said high degree of similarity observed between rat and human regulatory elements is illustrated by the partial sequence alignment attached as

ANNEX II

The rat sequence motifs are shown below the corresponding human sequence motifs. Identical nucleotide residues are identified by a vertical line between both sequences.

On the basis of said additional experimental evidence, I am convinced that a skilled person will not have to practice "trial and error" experimentation in order to provide

viral constructs other than those exemplified in the specification which can be successfully used for cardiac specific, in particular ventricle specific, gene transfer.

I would also expect that a similar favourable cardiac tissue specificity is also observed when the same regulatory elements are inserted in different viral vectors, because cardiac tissue specificity, which, in my opinion is the key for successful and valuable somatic gene transfer to the heart muscle, is mainly influenced by the cardiac tissue specificity of the promoter sequence operatively linked to the gene to be expressed with cardiac specificity.

In this respect, I would like to point to a comparative experiment already disclosed in the present specification. Example 11 describes the differences in cardiac tissue specificity and activity of gene expression observed on the one hand for a recombinant virus vector construct of the present invention, i.e. **Ad-mlcLuc** , and, on the other hand, for a virus construct carrying, **in the same virus, a different muscle derived promoter**, i.e. alpha-mhc. Said construct is designated **Ad-mhcLuc**. As illustrated by Figures 8A and 8B merely Ad-mlcLuc, i.e. the viral construct of the present invention, provides for a strong and cardiac specific gene expression while Ad-mhcLuc also directs non-specific gene expression. Significant levels of non-specific gene expression were observed for Ad-mhcLuc in kidney, spleen, liver, diaphragm, lung and intercostal muscle. Moreover, the construct of the invention was three to four times more active in the heart than Ad-mhcLuc. Said experimental evidence supports my point of view that a skilled reader of the present specification will in fact be able to practice, without the need of "trial and error" experimentation, the invention within the scope of the claimed recombinant vectors, carrying a regulatory mlc-2 gene fragment which must be functional, i.e. directs cardiac specific gene expression.

The undersigned declares further that all statements made herein on his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and

that such wilful false statements may jeopardise the validity of this application or any patent issuing thereon.

Munich, 15-06-2002

(Wolfgang M. Franz)

CAGTCTTGAG TGATGCTGAA AGGAACCCCT GAAGTCTACA AAGAACCAAG 50

TCCTCCCTGG ATTCTCCAAT CCCAGGGCCT TGTCCCTGGT CTTGGGGGCT 100

CCCTGGGGCA ACACAACCCA TTGATGAGAG AGACTTTGGA TCTCTGGCTC 150

TCTCAACAGA CCCCAAGGCC TAGTCCACAC CCCACGTGCT CCACGTCCCA 200

GCAGCCACGT GGTTCCATGC CCCATTCAGG CCGCCATTTC CCAGAATCCC 250

TAGACACAAT CTCACTTAAT CCTCCCAGCA GCCTTATGGA GGTGTGTGAT 300

CTCCCTTTTC CAGGTGAGGA AACAGGCCCG AGAGGGTGAG TGCCCTATTT 350

GACAACCCCT CTTGCTATCC AGCCAGAATG GTTCCTCTAG TACCCCTTCT 400

CCS-like sequence

GGAGGCCTGG CTGTACAGGT GTCCCTCAGG GACACACACC CCCACTCGAC 450

TCTGGGGGCC CAGCCCATCC TAATCCCCAC CCCGGGGCTT CCCACCCCCC 500

ATCATACACT CTCCACATCT TCTGTGGCTG CAACAACCTT TTCACTTGGC 550

CAGTTGGAGC TACTGACTGC TCACACAGGG TTTTAACGAA AATCTATGGT 600

GTGCCTATTA GCTAGGGAAA CATTTATTCT GGTGTTGTCA GAGAACCTTG 650

GACAGAAAAG CTCCTCTTGA TGTGTGCACT GCACATATGT GGATGCGTGT 700

ACATGCACGT GTCTGTGTGC CTCTATGCAT GTGCAGACGT GTTTTTGTCT 750

GTGCATGCAT GTGCCTACAC ACACACATGA ACACATCTTT TGTTATTAAA 800

GATCTGTCAG AAGAGTGTCC TGGGTAACTC TAACCCATGT GGGACTGCAG 850

AGAAGAAAAA AACCCACACC TTTTTTTGTC ACAGCCATCA ATGGTCCTTG 900

GGTTTGTGTG CCCCCAAATT GAGATTATTT TTCCACCTGA GAAGGGGAGT 950

GAGTGATAGC TACCATTTGC CAGGTCTCAC CTCCTTTTAC CCTCTGGAAA 1000

ACCTAATAAG AAAAGTGATT TCTTTTTTTA AGCTCTGGAA AACTCCAGCC 1050

CCAGGGGGCC TTCTGTTCCT CAAAGCCTCC AAATTCTCCC TGCCTTGAGG 1100

TATGTGCTGT CCCCACTGCC TGGAGCCCCC TTTGCAGACT CTGCTTGGAG 1150

GAGCCCACCT GCGCCCCTGT CTGAGGCTGT CACCTGGCCA CTGCCATGCC 1200

TCTGTCTCAT CCCTGCATGA GATCCGTCAC TGCCTGCAAC TGTCTGGGTT 1250

GTGCATTTGT TTACTTTCTC CTTGTCCATC TTCCCCTCGC ACTTACGCAC 1300

CTCAAGGGAA GGGAATTTGT TGCTTTGCGC TGGGCTCGAT GAAGGGGAAT 1350

GAATGCTGGT TCAGCCATCA GCCCCGCACC CACACTACTG GGAGGGCAGA 1400

GGGACATTCT CCTTCTTAGA GGTGTGGCCT CTGGCACTCA GGCCTGCCAC 1450

CCACGGACAC TAAATAACCA CAATGATTCC AAGCCCCGAG TTCTTGCTCC 1500

CTGAATCCCA AGGCTGTCTT TAAGGGCACA GGAAGATGGC CATCTTTTGT 1550

TGTTTTGGTT TAGTTTGGGG TTTTTTTGGT CTTTGTTTTT GAGATGGAGT 1600

CTTGCTCTGT CGCCCAGGCT GGAGTGCAAT GGCACGATCT CGGCTCACTG 1650

CAACCTCCGC CTCCTGGGTT CAAGCAATCC TCCTGCCTTA GCCTCCCAAG 1700

TAGCTGGGAG TACAGGCATG TGCCACAACG CCCAGCTAAT TTTTGTATTT 1750

TTAGTAGAGA TGGGGTTCTG TCATGTTGGC CAGACTGGTC TTGAACTCCT 1800

GACCTCAGGT GATCTACCCG CCTCGGCCTC CCAAAGTGCT AGGTGTGAGC 1850

CAACATGCCC GTCCTTTTTT TTTTTTTTTT TTTTTTTGAG TCAGAGTCTC 1900

ACTCTGTCGC CCAGGCTGGA GTGCAATGGC GCTATCTCGG CTCACTGCAA 1950

CCTCTGCCTC TCGGCCTCAA GCGATTCTCC TGCCTCAGTC TCCTGAGTAG 2000

CTGGGACTAC AGGCCCGCGC CACAACGCCT GGCTAATTTT TGTATTTTTA 2050

GTAGCGACAA GTTTCATCAT GTTGGCCAAG GTCGTCTTGA ACTCCTGACT 2100

CAAGTGATCC ACCCGCCTCA GCCTCTCAAA GTGCTGGCAT TTCAGGTATA 2150

AGCCACTGCA CCCAGCAGGA AAGCTGTCTT CAGTAAAAGT ATTATATAAT 2200

GACACCTTGC ATTCTGAGAG CAGCTGCTGT TTTCAAGGCT CTTAAAGAGC 2250

CTGGACTCTG GAGACAAAGG GGCCTCCAGA GGGGTCCACG CCTAGCTCCA 2300

TCACTGTGTG ACCCTGGGCA GCTCACTTCG CCTCTCTGAG CTTTTGTTTC 2350

CGCATCTGTA AAATGGGGGC ATGGATGATG AGGTGGTCCC CACCCTCTAG 2400

GGTGGCTGGA AAATTATGTG TGGGAGCCAT GAGCACATAG TGTCCGGCAC 2450

GTGCCAGTGC TCAGTCAATG AGATTTGTCA TTTCTTCAGT CAACAAATAT 2500

TTATTTTTGA GCTGCTGCTG TGTGCATCAT GAGCTGGGAG CTGGGGAGAC 2550

AGTCAGTGGT GAGGGAAACT AAAGTGATCC CTGCCCTCTG AGCTGACGCT 2600

CCACAGATGC TGAAGAAAAT GAGTCAGTGC ACTGTGGGCA GTGTTCGGGA 2650

CTGCCTCACG CTGTGCAGAG AAACAAAGAA GGGAGATCGG AGCGCAGGAG 2700

GTGCGTGGCT GTGTTATTTG TTTGTTTTGA GACAGGGTCT TGCTCTGTCA 2750

CCCAGGCTGG AGTGCAGTGG TGTGATCGTG GCTCACCACA GCCTCAACCT 2800

CCCGGGCCCA AGTGATCCTC CTGTCCCAGC CTCCTGAATA GCTGAGACTA 2850

TAGGCATGCA CCACCACGCA CAGCTATTTT TTTTTCTTTT GCGTAGAGAC 2900

AGGCATCTCC CTATGTCACC CAGGCTGGTC GCAAACTCCT AGGCTCAAGC 2950

AATCTTCCCG CCTCGGCCTC CCGCCGTGCT GGGATTTCAG GCATGAGCCA 3000

CAGTGCCAGC CTTCATGGTT ATTTTAAAGA TGGTGGTCGG GGAGGCTTCA 3050

CTCAGGAGAT GACATATGAG CAAAGATGCA GTGAAGGAGG TGAAGGAAGG 3100

AGCCGTGCGA TGACTGACAG AAAGACATTC CAGGTAGAGG GCACACAGGT 3150

GCAAAGACCC TGAGGCCAGA TCCAGGCTGA TAAAACAGAG CATTTTAGCA 3200

GTCTCCTCTC CCTGCCATTT TTTTTCTCAA AATTGACAAG CACAAGTGTC 3250

CCCGGCCCAA GCACCGCAGA GAGCGCGCAG CATCTCTCCC CGTGACCATG 3300

HF-3 element MLE1 element

ACCCAGCTAC TGCCTCTTTA ACCTTGAATG CCTTTTTGGG GGCTCACGTG 3350

HF-2-Box

TCACCCAGTG GCGAGTGAGC CACCCTTACT TCAGAAGAAC GGCATGGGGT 3400

E-Box HF-1a u.

GGGGGGGCCT TAGGTGGTGC CCGCCTCACC TATGACTGCC AAAAGCGGTC 3450

HF-1b-Box

ATGGGGTTAT TTTTAAACAT GGGGAGGAAG TATTTATTGT TCCTGGGCTG 3500

CAGAGAGCTG GGCGGAGTGT GGAATTCTTC TCGGGAGGCA GTGCTGGGTC 3550

CTTTCCACCA TG

CSS-1ik sequence

```
CAGGGACACACACCCCCACTCGACTCTGGGGGCCCAGCC-CATCCTAAT human
||||||||  |       |||     |||||  |||||||| |  |||   |||
CAGGGACAGAT-----CAC----CTCTGTGGGCCCAGACTCATAGTAAC rat
```

HF-3- and MLE1 elements

```
CTCTT TAACCTTGAATGC CTTTTTGGGGGC TCACGTGTC-A CCCAG human
      ||||||||||  ||               |||||||||  |
CTCTT TAACCTTGAAGGC ATTTTTGGGT-C TCACGTGTCCA CCCAG rat
        HF-3-Box                   MLE1 Box
```

HF-2-Element, E-Box, HF-1a- and HF-1b- elements

```
TGAGCCACC CTTACTTCAGAAGAACGGC ATGGGGTGGGGGGGCCTTAGGTGGTG human
         ||||||||||||||||||||
TGAACGGCT CTTACTTCAGAAGAACGGC ATGGGGTGGGGGGGC-TTAGGTGGCC rat
             HF-2-Box

CCCGCCTCACCTA TGACTG CCAAAA GCGGTCATG GGGTTATTTTTA human
              ||||    |  |||||||  ||||||||||||
TCTGCCTCACCTA CAACTG CCAAAA GTGGTCATG GGGTTATTTTTA rat
             E-Box       HF-1a-Box   HF-1b-Box
```